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CONTRACTING ORGANIZATION: MCPHS University
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project continues our work in the investigation of acupuncture as a treatment for the symptoms of Gulf War Illness. The goals of this current application are:1) Gather follow up data from our veteran participants (from our completed parent study *The Effectiveness of Acupuncture in the Treatment Of Gulf War Illness* W81XWH-09-2-0064) on current symptom levels and use of services to explore the long-term effects of an acupuncture treatment program, 2) Develop suggestions for how an acupuncture program may be implemented using the viewpoints of multiple stakeholders, 3) Develop a treatment manual for acupuncture practitioners explaining the most effective methods of treating the symptoms of GWI. As planned in the Statement of Work, we have completed the tasks: 1. Create program evaluation (follow up veteran survey) documents, 2. Train research assistant, and review goals with consultants, 3. Complete IRB Review, 4. Begin program evaluation with study subjects (in process), 5. Plan Program evaluation with multiple stakeholders (in process), 6. Start Delphi process.

15. SUBJECT TERMS
Gulf War Illness, Complex Medical Illness, Acupuncture, Treatment Trial, Secondary Date Analysis

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Number of Phone Calls	134
Number of Mailings: Letters/flyers/Consent Forms	348
Number of Emails	104
Number of Veterans now Deceased	2

Providing further detail Table 1, 73 Informed Consents were sent out; sometimes a consent was sent more than once to unresponsive veterans. Forty-four Informed Consents were completed and returned to us and these same subjects were sent surveys to fill out. Out of this 44 total, 36 completed the survey, 6 have yet to do the survey, 1 opted out, 1 no longer wants to be contacted.

Once contacted, subjects are mailed an Informed Consent and IRB approved survey which: (1) repeats the survey questions administered in the parent study, (2) asks additional questions about subjects' study experiences, use of health services since the study, and requesting feedback and suggestions for program and treatment improvement.

Subject contact has been slow, thus we have implemented some additional techniques to resurvey our sample. We started asking veterans we contacted what they would like as incentive. We were told that they wanted more acupuncture. Our host institution (New England School of Acupuncture) agreed to provide 5 free treatment vouchers to our student clinic in Newton MA, and Worcester MA. This information was shared in a mailing to subjects in June. Two subject responses came from this. We plan to repeat this with those that did not answer on Nov 1. Currently, a fellow Boston GWI researcher (Kim Sullivan at Boston University) has sent us leftover incentive materials (commemorative coins and Desert Storm tee shirts) and we will begin sending those to our remaining sample November 1, 2017 as part of our next attempt to reach the subjects.

If this technique fails, then we will attempt a shortened phone survey to gather at least the main outcomes from the subject using questions over the phone.

Task 4.2.4 Conduct Delphi process (Month 18-29). The Delphi process began with the initial treatment questions being drafted by collaborator Rose Schnyer DAOM. The 5 (from a complete sample of 31) treating practitioners from the parent study with the best treatment responses and who agree to participate came together with the named members of our study team on the afternoon of November 20th, to review those questions.

Task 4.2 Program evaluation with multiple stakeholders (Month 11-22)
We executed the first set of meetings November 19th and 20th 2016. We decided to begin this process before the scheduled date (per the Statement of Work) as the re-surveying of the veteran-study subjects is proving more time consuming than planned.

This Practitioner Meeting took please Saturday, November 19, 2017 – Sunday November 20, 2017 at the New England School of Acupuncture, 150 California St. Newton MA 02458. The purpose of the meeting is to connect with the Practitioners from the Parent Grant to discuss the development of a Treatment Program for Gulf War Illness with the Acupuncture Consultants. This meeting followed a survey we conducted of Acupuncture Practitioners who participated in the parent grant. The group reviewed survey results from the Practitioners and discussed strategies for development of program.

Attendance:

Lisa Conboy, Director of Research
KaiYin Hsu, Research Assistant

Grant Consultants
Lisa Taylor Swanson, Lic.Ac., School of Medicine, University of Utah
Joe Chang, Lic.Ac., Chang Acupuncture
Dr. Iris Bell, College of Medicine, Tuscon, University of Arizona
Dr. Marc Goldstein, VA Central Western Massachusetts

Study Veterans (veterans from our parent study)
Tara Batista
Jay Pinette
Pat Hoarty
Gareth Mannion

Study Acupuncturists (practitioners from our parent study)
Dinah Shatz
ZhenZhen Zhang
Christine Lee
Eva Lunetta
Diana Dia

Veteran Advocates
Dee Lane, Campaign for Military Families
Dr. David Chen, Edith Nourse Rogers Memorial *Veterans* Hospital, *Bedford, MA*

What opportunities for training and professional development has the project provided?
Not Applicable/Nothing to Report.
How were the results disseminated to communities of interest?
In addition to published articles and conference proceedings mentioned below, our parent paper results were posted to **five** Facebook social media groups that support veterans, Gulf War Illness specific information, or acupuncture: 1) *Acupuncturists on Facebook* (readership 11,958), 2) *Acupuncture researcher share group* (readership 8,125), 3) *Gulf War Illnesses Facebook Group* **(closed group/invitation only,** readership 11,253), 4) *Gulf War Illness-Save Our Service Members Facebook Group* **(closed group/invitation only,** readership 4,965), 5) *Gulf War Illness Veterans Support Group* **(closed group/invitation only,** readership 280).

Parent study results were also posted twice to the Gulf War Illness information cite *91 outcomes* 2015, 2017. (http://www.91outcomes.com/). 91outcomes.com is a health and news website for veterans of the 1991 Gulf War.

In addition, *our study team informally shared our results with Veteran Administration contacts (e.g. Stephanie Taylor PhD, Associate Director, VA HSR&D Center for the Study of Healthcare Innovation, Implementation & Policy, VA Greater Los Angeles Healthcare System*), other veteran researchers (e.g. *Kim Sullivan PhD at Boston University's CDMRP-funded Gulf War Illness Consortium*), and the veteran advocacy groups (e.g. *Campaign for Military Families).*

start discussions about NESA collaborating with this local VA to establish a student clinic on site, which would offer treatment to veterans on site at no cost to the VA or the veteran patients.

We will also continue to: 1) publish updates on social media, 2) Collaborate on projects with GWI collaborators, 3) continue outreach to our parent study sample, 4) complete the associated manuscripts (Appendix A).

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
The results of this project will allow for a smooth implementation of an acupuncture treatment program for veterans, active military personnel, and the general citizen. This information will be most easily applied by, and results will be supplied to, the Department of Veterans Affairs.

What was the impact on society beyond science and technology?
Our results have the potential to inform medical decision making in support of acupuncture as a viable treatment for veterans with GWI.

5. CHANGES/PROBLEMS

We have requested and received a 6 month no-cost extension to continue our recruitment efforts for the resurvey aspect of this project (Task 4.1). We have implemented some additional techniques to resurvey our sample this past year. We started asking veterans we contacted what they would like as incentive. We were told that they wanted more acupuncture. Our host institution (New England School of Acupuncture) agreed to provide 5 free treatment vouchers to our student clinic in Newton MA, and they agreed that we could tell subjects that treatments at our Worcester teaching clinic are free. This information was shared in a mailing to subjects in June. Two subject responses came from this. We plan to repeat this with those that did not answer on Nov 1. Currently, a fellow Boston GWI researcher (Kim Sullivan at Boston University) has sent us leftover incentive materials (commemorative coins and Desert Storm tee shirts) and we will begin sending those to our remaining sample November 1, 2017 as part of our next attempt to reach the subjects. If this technique fails, then we will attempt a shortened phone survey to gather at least the main outcomes from the subject using questions over the phone.

6. PRODUCTS:

Journal publications. *All listed show acknowledgement of federal support.*

Screening for novel central nervous system biomarkers in veterans with Gulf War Illness.
Abou-Donia MB, Conboy LA, Kokkotou E, Jacobson E, Elmasry EM, Elkafrawy P, Neely M, Bass CR, Sullivan K. Neurotoxicol Teratol. 2017 May;61:36-46. doi: 10.1016/j.ntt.2017.03.002. Epub 2017 Mar 9. PMID:28286177. Published.

How TCM Practitioners Treat Gulf War Illness; findings of an RCT with individualized treatments. Poster Presentation. Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Rosa Schnyer DAOM, Lisa Conboy MA MS ScD. Society for Acupuncture Research International Symposium. April 27-29, 2107, San Francisco, CA.*

Treating Complex Veteran Illness with Acupuncture in the Community. Oral Presentation. Lisa Conboy MA MS ScD, Kai Yin Hsu Lic Ac, Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM. Society for Acupuncture Research International Symposium April 27-29, 2107, San Francisco, CA.

Development of Therapeutic Alliance in Acupuncture Treatments in a Veteran Population Poster Presentation. Saadat Bagherigaleh, MD, Lisa Conboy MA MS ScD. Society for Acupuncture Research International Symposium April 27-29, 2107, San Francisco, CA.*

Management of Gulf War Syndrome Symptoms with Acupuncture: Findings of a Wait-list Controlled RCT. Lisa Conboy MA MS ScD. Oral Presentation to Center for Healthcare Organization and Implementation Research (CHOIR), June 9, 2017. Jamaica Plain VA Campus Boston, MA.

Website(s) or other Internet site(s):
Posted to Facebook social media groups that support veterans, Gulf War Illness specific information, or acupuncture: 1) *Acupuncturists on Facebook* (readership 11,958), 2) *Acupuncture researcher share group* (readership 8,125), 3) *Gulf War Illnesses Facebook Group* (readership 11,253), 4) *Gulf War Illness-Save Our Service Members Facebook Group* (readership 4,965), 5) *Gulf War Illness Veterans Support Group* (readership 280).

Posted twice to the Gulf War Illness information cite *91 outcomes* 2015, 2017. (http://www.91outcomes.com/). 91outcomes.com is a health and news website for veterans of the 1991 Gulf War.

Spoke about study on Radio Program "Warrior Connection" with Patricia Axelrod, Wednesday Oct 4, 2017.

Technologies or techniques Nothing to Report
Inventions, patent applications, and/or licenses Nothing to Report
Other Products Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Lisa Conboy*
Project Role:	Principle Investigator

Name:	Beth Ann Schmitt
Project Role:	Research Project Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.2
Contribution to Project:	Beth Ann Schmitt has assisted with recruitment. She has participated in regular meetings with her PI and consultants.
Funding Support:	N/A

Name:	Rosa Schnyer
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Rosa Schnyer was lead consultant on the project. She participated in hypothesis generation and manuscript preparation.
Funding Support:	N/A

Name:	Joe Chang
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Joe Chang assisted with categorizing acupuncture protocols and manuscript preparation.
Funding Support:	N/A

Name:	Dr. Marc Goldstein

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Dr Lisa Conboy

Appendix A

Publications

Neurotoxicology and Teratology 61 (2017) 36–46

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Full length article

Screening for novel central nervous system biomarkers in veterans with Gulf War Illness

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ABSTRACT

Gulf War illness (GWI) is primarily diagnosed by symptom report; objective biomarkers are needed that distinguish those with GWI. Prior chemical exposures during deployment have been associated in epidemiologic studies with altered central nervous system functioning in veterans with GWI. Previous studies from our group have demonstrated the presence of autoantibodies to essential neuronal and glial proteins in patients with brain injury and autoantibodies have been identified as candidate objective markers that may distinguish GWI. Here, we screened the serum of 20 veterans with GWI and 10 non-veteran symptomatic (low back pain) controls for the presence of such autoantibodies using Western blot analysis against the following proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated tau proteins (Tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP), calcium-calmodulin kinase II (CaMKII) and glial S-100B protein. Serum reactivity was measured as arbitrary chemiluminescence units. As a group, veterans with GWI had statistically significantly higher levels of autoantibody reactivity in all proteins examined except S-100B. Fold increase of the cases relative to controls in descending order were: CaMKII 9.27, GFAP 6.60, Tau 4.83, Tubulin 4.41, MAG 3.60, MBP 2.50, NFP 2.45, MAP-2 2.30, S-100B 1.03. These results confirm the continuing presence of neuronal injury/gliosis in these veterans and are in agreement with the recent reports indicating that 25 years after the war, the health of veterans with GWI is not improving and may be getting worse. Such serum autoantibodies may prove useful as biomarkers of GWI, upon validation of the findings using larger cohorts.

1. Introduction

Approximately one third of the 697,000 US military personnel who served in the Gulf War (GW) from August 1990 to June 1991, have reported persistent symptoms for many years after the war (RAC, 2008; IOM, 2012, RAC, 2016; White et al., 2016). This complex of symptoms, known as Gulf War Illness (GWI), include memory and attention problems, profound fatigue, chronic muscle and joint pain, severe headaches, persistent diarrhea, respiratory difficulties and skin rashes. GWI is primarily diagnosed by symptom report and no validated objective diagnostic biomarkers currently exist that fully segregate cases from controls. This study was designed to identify objective central nervous system (CNS) biomarkers of GWI using clues from prior clinical studies with GW veterans and from animal studies that modeled chemical exposures experienced by GW veterans.

Clinical studies have reported impaired cognitive functioning and reduced MRI volume and altered white matter microstructural integrity in organophosphate (OP) pesticide, sarin nerve agent and pyridogstigmine bromide (PB) anti-nerve gas pill-exposed GW veteran cohorts (White et al., 2016; Sullivan et al., 2013; Chao et al., 2010;

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0892-0362/

proteins (Abou-Donia et al., 2013; Banks and Lein, 2012; Golomb, 2008; Terry, 2012; Binukumar and Gill, 2010; Soltaninejad and Abdollahi, 2009). In this study, we determined circulating IgG- class autoantibodies in serum from 20 GWI cases and 10 symptomatic (low back pain) controls against the following 9 brain proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated protein-tau (tau proteins), microtubule associated protein-2 (MAP-2), calcium/calmodulin Kinase II (CaMKII), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP) and S-100B.

2. Materials and methods

2.1. Materials

The sources of proteins were: NFP (bovine spinal cord), tau protein (human), MAP-2 (bovine serum), tubulin (bovine brain), and MBP (human brain), from Sigma-Aldrich (Saint Louis, Missouri); CaMKII (Human) recombinant Protein and MAG recombinant Protein from Novus Biologicals, Littleton, CO, GFAP (human) from Biotrend Chemikalien GmbH, (Cologne, Germany) and S-100B (human brain) from American Qualex International, Inc. (San Clemente, California). Horseradish peroxidase-conjugated goat anti-human IgG, and enhanced chemiluminescence reagent were obtained from Amersham Pharmacia Biotech (Piscataway, New Jersey). SDS gels, 2–20% gradient (8 × 8), and tris-glycine 15 mM were obtained from Invitrogen (Carlsbad, California). All other materials were purchased from Amersham.

2.2. Ethics statement

Approval for the use of stored blood samples for this study was obtained from the Duke University Medical Center Institutional Review Board.

2.3. Case and control samples

Serum samples from 20 GWI cases with GWI and 10 non-veteran symptomatic controls with lower back pain were tested in this pilot study. GW veteran serum samples were collected from a study of acupuncture treatment in veterans with GWI from 2010 to 2012 (Conboy et al., 2012). Control serum samples were derived from a separate study of non-veteran patients with chronic lower back pain who served as 'symptomatic low back pain' controls from 2011 to 2013 (Jacobson et al., 2015). Veterans with GWI will be referred to as 'cases' and low-back pain symptomatic controls will be referred to as 'controls'.

2.4. Description of the patient cohorts

2.4.1. GWI-case cohort

"The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" PI: Conboy, (8/21/2010–12/26/2012) N = 104; Study Site: New England School of Acupuncture (NESA). Cases were recruited through the Defense Manpower Data Base (DMDC) personnel listings and advertisements. Cases were screened for GWI symptoms and were required to meet the CDC diagnostic criteria for chronic multi-symptom illness (CMI) in order for inclusion in the parent study and in the current study (Conboy et al., 2012; Fukuda et al., 1998). Inclusion in the current study also required that veterans were deployed to the 1990–1991 Gulf War. CMI is characterized by one or more symptoms of at least 6 months duration from at least two of three symptom categories: 1) fatigue; 2) mood-cognition; 3) musculoskeletal pain.

Symptoms were not necessarily required to have started during or after the Gulf War deployment. Exclusionary criteria included that the veteran was 1) currently enrolled in another clinical trial 2) Had another disease that likely could account for the symptoms, as determined by the Medical Monitor 3) Severe psychiatric illness (in the last 2 years psychiatric hospitalization, suicidal attempt, alcohol or substance abuse, use of antipsychotic medication) 4) Unable to complete the protocol based on the evaluation of the Medical Monitor.

2.4.2. cLBP-cohort

"Structural Integration for chronic low back pain" PI: Jacobson (3/4/2011–6/21/2013) N = 46. Study Site: Spaulding Rehabilitation Hospital (SRH). In this cohort, 46 outpatients from the Boston area with chronic nonspecific low back pain were randomized to parallel 20-week long treatment groups of structural integration (SI) plus outpatient rehabilitation (OR) versus OR alone. The details of the study are described in a recent publication (Jacobson et al., 2015). Inclusion criteria for the parent study included: (i) Men and women aged 18–65, (ii) cLBP of ≥6 months duration, not attributed to infection, neoplasm, severe radiculopathy (as indicated by frequent severe pain radiating down a leg), fracture, or inflammatory rheumatic process, (iii) bothersomeness of back pain self-rated on average over the preceding 6 months ≥3 on an 11-point ordinal scale (0 = none, 10 = worst imaginable), (iv) prior arrangement to enter a course of outpatient physical therapy for low back pain at a Boston area rehabilitation clinic, (v) English language fluency and mental capacity sufficient to provide informed consent and participate in the study. Exclusion Criteria for the study included: (i) Impaired hearing, speech, vision, and mobility sufficient to interfere with participation in the study, (ii) current or anticipated receipt of payments from Worker's Compensation or other insurance for disability attributed to low back pain, (iii) prior treatment with any SI therapy, (iv) plans to initiate additional treatment for back pain during the period of the study other than outpatient rehabilitation care, particularly massage or other manual therapies (e.g., chiropractic or osteopathic manipulation), (v) exclusions for safety: unresolved musculoskeletal pathology of the lower limbs, current pregnancy, any implanted medical device, osteoporosis, any hypercoagulation condition, eczema, skin infection, deep vein thrombosis, burns or other acute trauma including unhealed bone fractures or open wounds, psoriasis, psychiatric illness not well controlled, or current episode of exacerbated major depressive disorder.

2.5. Collection and storage of samples

Samples from the GWI-cohort and the cLBP-cohort were all collected from the Boston area at the same time period at two different sites from 2010 to 2013. All sites followed exactly the same protocol for venipuncture, blood handling, serum separation, aliquoting and storage at −80 °C. The same phlebotomy and sample protocol was distributed in writing to all sites. All samples analyzed were baseline blood samples collected pre-intervention therapy. Samples used for this study have not been previously thawed and are free of hemolysis by visual inspection (Tuck et al., 2009).

2.6. Participant demographics

The participant demographics indicate that a total of 20 veterans with GWI, 18 males and 2 females, compared to 6 females out of 10 cLBP controls participated in the study. The age of the GWI cases ranged from 38 to 61 (mean ± SD 46.0 ± 6.8) compared to 25 to 64 (mean ± SD 50 ± 11.4) years for controls; all study participants were white (Table 1). Seventy percent of veterans with GWI reported taking PB

Table 1
Study participant demographics.[a]

Demographics	Cases	Controls
Age (mean ± SD)	46 (6.4)	50 (11.4)
Gender (% female)*	10	60
Race (% Caucasian)	100	100

Age range of Cases = 38–61 years and Controls = 25–64 years in 2010–2013 when the blood was collected.

[a] A total of 20 cases and 10 controls participated in the study.

* Cases were significantly different from controls for gender $p < 0.05$ but not for age.

Table 2
Chemicals, environmental and other exposures of cases during the Gulf War.[a]

Chemical exposures			Environmental and other exposures		
	Exposed	%		Exposed	%
Pyridostigmine bromide (PB)	14	70	Khamisiyah notification letter	8	40
Organophosphorus pesticides (OP)	7	35	Contaminated food/water	18	90
Carbamates	7	35	Vaccines	18	90
Pyrethroids	4	20	Malaria	12	60
DEET	11	55	Sand	18	90
Sarin	9	45	Tent heater	11	55
Depleted uranium (DU)	6	30	Jet fuel	14	70
Solvents	10	50	Oil fires	18	90

[a] A total of 20 veterans with GWI participated in the study.

calculated. Pairwise correlations among the nine biomarkers were assessed. A 2-sided p value <0.05 was considered significant. Due to the exploratory nature of this pilot study, analyses were not adjusted for multiple comparisons.

3. Results

As previously described, we assessed the specificity of the serum autoantibody by performing peptide/antigen competition assay, in which the serum was spiked with the target protein or peptide. The serum bound to tau eliminated the tau band in the Western blot (see Fig. 1) while the band of MAP-2 or MBP were present and not affected. The serum bound to MAP-2 eliminated the MAP-2 band in the Western blot while the band of tau or MBP was present. The serum bound to MBP eliminated the MBP band in the Western blot while the bands of tau and MAP-2 were present. These results indicate that each autoantibody in the serum was specifically neutralized by its target protein in serum sample and was no longer available to bind to the epitope present in the protein on the Western blot. This confirmed that the assay used in this study, was specific and accurately determined autoantibodies against tested proteins in serum samples.

To detect autoantibodies in serum, we probed Western blots with individual serum samples. A total of 30 human serum samples (20 veterans with GWI and 10 non-veteran symptomatic low-back-pain controls) underwent measurement of the levels of the serum circulating IgG-class autoantibodies against nine neuronal– and glial- specific proteins. Table 2 lists the number of GWI cases who were exposed to chemical and environmental exposures. It shows that 14 cases (70%) used PB as a prophylaxis against possible exposure to nerve agents and nine cases reported being exposed to the nerve agent sarin. In addition, a total of eight cases reported receiving notification from the Department of Defense (DOD) that they were potentially exposed to sarin and other chemicals due to their proximity to the Khamisiyah, Iraq underground weapons depot where a chemical weapons cache was destroyed in March 1991 (US DOD, 2002). Eight cases reported exposure to depleted uranium. All of the cases reported exposure to one or more insecticides or a mixture of pesticides including organophosphates, carbamates, pyrethroids and organochlorines. Eleven cases used the insect repellant DEET. All cases underwent environmental and other exposures listed in Table 2. Other chemicals that the cases reported exposure to included oil well fires, sand, tent heaters, jet fuel, and solvents. Some veterans reported exposure to malaria and 18 reported being vaccinated. Serum from GWI cases showed significantly increased levels of autoantibodies against all cytoskeletal proteins except those against S-100B compared to non-veteran symptomatic (low back pain) controls (Table 3). Due to the gender differences between the cases and controls, analyses were also run with just the males in the groups. Although there was only a small number of males ($n = 4$) in the control group which could be problematic in this type of analysis, results of this comparison showed a very similar pattern of significant differences in all autoantibodies (GFAP $p < 0.001$; Tau $p < 0.001$; MAP $p < 0.002$; MAG $p < 0.001$; PNF $p < 0.006$; Tubulin $p < 0.003$; MBP $p < 0.01$; S-100B $p = 0.31$). The majority of GWI serum reacted intensely to neural proteins, while most control serum showed a weak or no reaction. Fig. 1a and b present Western blots results from three representative GWI cases and three controls. The levels of serum autoantibodies in GWI cases and controls to neural-specific proteins expressed as mean values ± SD of triplicate assays of optical density arbitrary units normalized to total serum IgG optical density ranged from 0.30 for S-100B and 4.09 for GFAP for the cases compared to 0.30 and 0.62, respectively for controls are listed in Table 3 and shown in Fig. 2. The percentage of autoantibodies against neural proteins of cases compared to controls (in descending order) were: CaMKII, 927, GFAP 660, Tau 483,Tubulin 441, MAG 360, MBP 250, NFP 245, MAP-2 230, S-100B 103. Fig. 3 presents the mean values ± SD ($p < 0.001$) of fold increase of autoantibodies against neural proteins for the cases compared with the controls. Serum from controls had no or low levels of circulating autoantibodies to nervous system-specific biomarkers. Autoantibodies against CaMKII were more predominant in the cases' serum than in controls' serum (Fig. 3).

Fig. 4 shows that Tubulin and GFAP had the highest values in the GWI cases compared with the controls. Pairwise correlations among the nine autoimmune biomarkers were significant only for the pair Tau and MBP. When comparing the correlation between each pair, only tau and MBP were significantly linearly correlated to each other (Fig. 5). Fig. 5 shows that the control values of those two biomarkers were <1 optical density unit, whereas GWI cases had values strongly linearly correlated with each other such that on average tau was elevated up to 10 times higher than controls in some GWI cases, and MBP was also elevated up to 5 times higher for the same cases vs the controls.

Finally, when each biomarker was compared separately between individual cases and controls for potential fold-increase cut-points to discriminate the groups, results indicated that tubulin values had some of the highest-fold increased values in the individual GWI cases compared with the individual control values although only 60% of the individual cases ($n = 12$) showed that effect (Fig. 6a). However, in 9 (out of the 20) cases tubulin values were elevated by a factor of 3 to 9-fold higher than the controls. In Fig. 6b, GFAP was elevated the most in cases compared to controls. In fact, GFAP was higher in all of the cases compared with all of the controls with 20 out of 20 cases having 2 to 7 fold higher

Table 3
Statistical analysis of the levels[a] of serum autoantibodies (AA) in controls[b] and GWI cases[b] to neural-specific proteins.

	NFP	Tau	Tubulin	MBP	MAG	MAP2	GFAP	S-100B	CaMKII
Cases Mean ± SD	1.42±0.24	2.52±0.31	3.48±0.78	1.75±0.30	1.44±0.28	2.18±0.29	4.09±0.33	0.30±0.03	1.02±0.20
Controls Mean ± SD	0.58±0.09	0.60±0.09	0.79±0.11	0.70±0.11	0.40±0.04	0.086±0.09	0.62±0.11	0.29±0.04	0.11±0.03
p values	0.02	0.0001	0.001	0.001	0.007	0.002	0.00001	0.40	0.015

[a] The results are expressed as mean values of ± triplicate assays of optical density arbitrary units normalized to IgG optical density as fold of healthy controls.
[b] Values from cases were compared to the control group using *t*-tests; most were highly significant $p < 0.001$ (2-sided), except for S-100B that was not significantly different from controls. Cases were significantly different from controls with respect to gender $p < 0.05$ but not with respect to age.

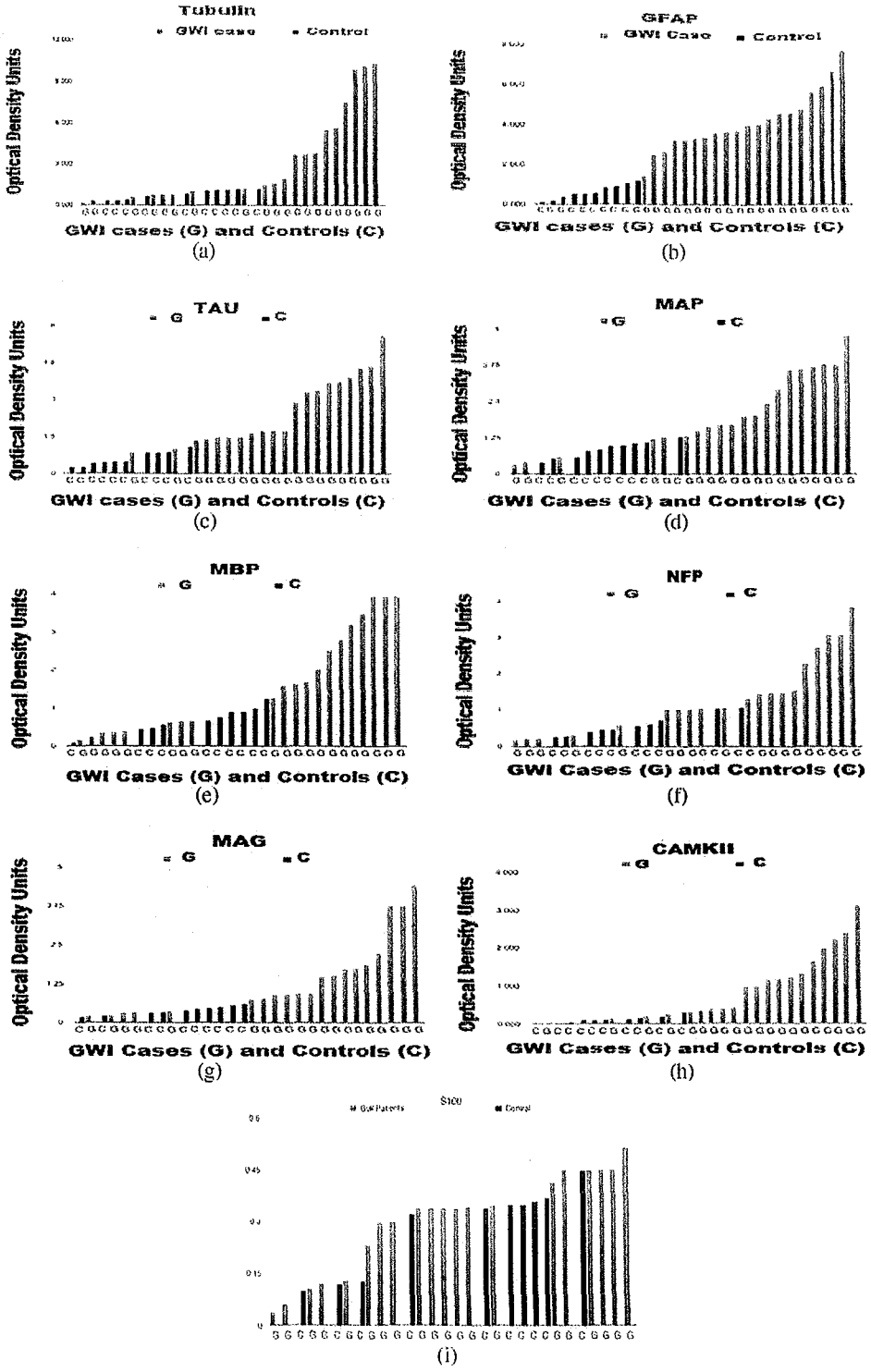

Fig. 6. a) Tubulin levels were higher than all controls in 12/20 cases. b) GFAP levels were higher than all controls in 20/20 cases. c) Tau levels were higher than all controls in 17/20 cases. d) MAP levels were higher than all controls in 15/20 cases. e) MBP levels were higher than all controls in 12/20 cases. f) NFP levels were higher than all controls in 10/20 cases. g) MAG levels were higher than all controls in 15/20 cases. h) CAMKII levels were higher than all controls in 16/20 cases. i) S100B levels overlap with cases and controls.

brain-blood barrier induced by astrocyte alterations. Waste proteins in the brain ultimately reach the liver through a mechanism known as the "glymphatic system" where they are degraded (Nedergaard, 2013). However, the released proteins that could serve as markers of injury are present in the short-term and cannot be used as biomarkers in the case of chronic GWI (Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014). Thus detection of autoantibodies can serve as surrogate markers for these circulating waste proteins as described in this study.

The highest increase in autoantibodies was against CaMKII which was 9.27 times higher than that of controls followed by GFAP which was 6 times higher than controls. This result is consistent with the veterans' exposure during their deployment to the Gulf War to

4.1. Limitations and future directions

This study, like all studies has important limitations. Although the present pilot study can serve as a proof-of-concept it has a small sample size and non-matched subject groups for age, gender and for CNS symptoms. This is particularly important as it has also been shown that CNS autoantibodies have been reported to be age-related in animal models (Lal and Forster, 1988). In addition, the convenience comparison group utilized in this study had musculoskeletal symptoms and not CNS symptoms therefore, it remains to be shown that these CNS autoantibody markers can clearly distinguish between GWI cases and additional groups with CNS specific symptoms. However, the strong results including 9-fold higher levels of CAMKII, 6-fold higher levels of GFAP and 4-fold higher levels of tau and tubulin that were presented in this study warrant further research for a blood-based objective marker of GWI in larger, well-characterized veteran cohorts. These results suggest a possible new avenue for further development of an objective biomarker of GWI. The identification of this small panel of neural-specific autoantibody biomarkers in GWI shows promise for further validation in larger study samples that are more carefully matched for subject demographics (particularly age), different types of control groups (i.e. healthy and CNS symptomatic groups) and that classify cases by both the CDC and the more specific Kansas GWI criteria which also specifies the time period of deployment which may be relevant to particular OP and other deployment-related exposures (Steele, 2000; Fukuda et al., 1998). Future directions will be to compare these CNS autoantibody markers with specific behavioral outcomes including cognitive performance and brain imaging of gray and white matter volume and microstructural integrity to further validate these suspected brain-immune-behavioral outcomes.

5. Conclusions

In conclusion, in this pilot study GWI was significantly associated with 2–9 fold increased serum autoantibodies against 8 neuronal- and glial-specific proteins (CaMKII, GFAP, Tau, Tubulin, MAG, MBP, NFP, MAP-2) and not with a marker of more acute damage (S-100B). The autoantibodies that were found here to be elevated in GWI, targeted proteins/ antigens that play critical roles in the structure and function of the neuron including axonal transport and myelination. Many of them are explicit markers for neurodegenerative disorders, consistent with axonal and myelin degeneration of myelinated neurons and with astrogliosis, cell signaling and neuroinflammation. These same proteins have been shown to be affected in other clinical groups and animal models with similar organophosphate and carbamate exposures (Abou-Donia et al., 2013, 2014). These results validate prior reports of increased MBP autoantibodies in GWI cases and suggest that oligodendrocyte signaling, glia and white matter alterations should continue to be further studied in GWI and validated with health symptom and behavioral outcomes (Vojdani and Thrasher, 2004). The results also indicate that veterans with GWI may be continuing to show brain neuronal degeneration and glial activation that would be consistent with recent reports of chronically persistent and in some cases worsening health of these veterans (Smith et al., 2013; Ozakinci et al., 2006; Li et al., 2011; Kang et al., 2009; Dursa et al., 2016; White et al., 2016). These results suggest a possible avenue for further development of a panel of objective biomarkers of GWI upon further validation in larger study samples that are more carefully matched for subject demographics.

Conflict of interest statement

The authors report no relationships that could be construed as a conflict of interest.

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Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm

By Joe C. Chang, MAOM, Dipl. OM, LAc, Rosa N. Schyner, DAOM, CFMP, LAc and Lisa Conboy, ScD

Clinical and registry programs indicate that 25 percent of the 700,000 veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990-1991), have been affected by clusters of symptoms, and co-morbid medical diagnoses. Symptoms include chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, post traumatic stress disorder (PTSD), and other anxiety disorders.[1,2]

Gulf War Illness (GWI) is a complex and difficult medical condition to treat, with highly individualistic symptom presentations, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain. The Centers for Disease Control and Prevention (CDC) has defined Gulf War Illness as three symptom clusters that includes: fatigability (fatigue 24 hours or more after exertion), mood and cognition (feeling depressed, irritable, anxious, difficulty in concentrating, problems getting to sleep), and musculoskeletal (joint or muscle pain).

GWI & Acupuncture

The complex diagnostic and treatment process of Chinese medicine, which is tailored to each individual's clinical presentation, can provide an effective framework for evaluating and addressing the complex constellation of symptoms presented in GWI. Currently, no biomedical standard of treatment care exists. One study, completed by the *New England School of Acupuncture,* included an unblinded phase II Randomized Controlled Trial (RCT), which offered individualized acupuncture treatments, using the available community resources.

The treatment schedule duration, dose, and specific Chinese medicine techniques employed were based on the clinical experience of the expert practitioners, and informed by literature review. Details of the protocol and implementation were determined before the trial began via focus groups with senior acupuncture faculty.

Case Study Team

Licensed acupuncturists with at least five years of clinical experience, who received additional in-house training concerning GWI, provided the acupuncture treatments. Although there are many styles of acupuncture within Chinese medicine, acupuncturists were chosen who self-reported use of the TCM model of diagnosis.

During the first session, the acupuncturists conducted an interview reviewing the subject's medical history, symptoms and aspects of diagnosis from the perspective of TCM, including condition of the tongue, pulse, meridians, and acupoints.

Each subject received an individualized diagnosis and treatment protocol addressing his or her unique pattern of symptoms. Brief interviews began each subsequent session, allowing patient and practitioner to prioritize

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Characteristics of Gulf War Illness participants in an acupuncture study

--Manuscript Draft--

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Article Type: Research Article

Full Title: Characteristics of Gulf War Illness participants in an acupuncture study

Short Title: Characteristics of GWI participants

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Keywords: Gulf War Illness, veteran characteristics, pain, sleep, mood, depression, anxiety, PTSD, unexplained symptoms

Abstract: BACKGROUND: Gulf War veterans reported significantly more often nonspecific multiple complex medical symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain.
METHODS: We analyzed baseline characteristics reported by Gulf War Illness (GWI) study participants. The data was from a pragmatic randomized clinical trial to test the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community. Veterans with diagnosed symptoms of GWI were included in the trial. This report focuses on sociodemographic characteristics, the SF-36 physical component scale score (SF-36P), and the McGill Pain scale at baseline.
RESULTS: Of the 192 participants assessed for eligibility, 104 subjects underwent randomization. Mean age was 48 years, 13% were female and over 2/3 self-described white in both groups. Over 2/3 reported diseases of the musculoskeletal system and connective tissue and over half reported symptoms, signs and abnormal clinical or laboratory findings. Over 1/3 of participants indicated that they were currently diagnosed with anxiety (36% of the sample), depression (35%) and Post Traumatic Stress Disorder (33%). Other concurrent diagnoses included sleep apnea (28%) and Chronic Fatigue Syndrome (28%).
DISCUSSION: Gulf War Veterans in the present study diagnosed with Gulf War Illness report many similar symptoms and diagnoses to GW veterans studied elsewhere. Specifically, increased mental health conditions such as depression, anxiety, and PTSD were identified in this sample, as well as physical conditions of Chronic Fatigue Syndrome and unidentified symptoms. Safe and effective interventions for these symptoms and condition need to be identified and studied for this population.
CONCLUSIONS: Further research is needed to identify and test safe and effective interventions for symptoms and conditions experienced by Gulf War veterans.

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Opposed Reviewers:

Additional Information:

Question	Response
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Ethics Statement

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Title: Characteristics of Gulf War Illness participants in an acupuncture study

Running Title: Characteristics of GWI participants

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Key words: Gulf War Illness, veteran characteristics, pain, sleep, mood

(35%) and Post Traumatic Stress Disorder (33%). Other concurrent diagnoses included sleep apnea (28%) and Chronic Fatigue Syndrome (28%).

DISCUSSION: Gulf War Veterans in the present study diagnosed with Gulf War Illness report many similar symptoms and diagnoses to GW veterans studied elsewhere. Specifically, increased mental health conditions such as depression, anxiety, and PTSD were identified in this sample, as well as physical conditions of Chronic Fatigue Syndrome and unidentified symptoms. Safe and effective interventions for these symptoms and condition need to be identified and studied for this population.

CONCLUSIONS: Further research is needed to identify and test safe and effective interventions for symptoms and conditions experienced by Gulf War veterans.

rheumatoid arthritis, seizures, coronary heart disease, migraine headaches, hypertension and asthma. Statistically significantly higher rates of mental health conditions in Gulf War Veterans compared to Gulf War era veterans included Post Traumatic Stress Disorder, major depressive disorder, anxiety disorder, and high somatic symptom severity. Gulf War veterans that rated their health as excellent (3.6%) or very good (10.3%) was significantly lower ($p<0.001$) than the proportion of Gulf Era veterans who reported their health as excellent (6.1%) or very good (16.2%).

The CDC defined GWI as having at least one chronic symptom from two of the following three areas: mood/cognition, fatigue, and musculoskeletal. (5) GWI is also a chronic multisystem condition that is significantly associated with deployment to the Gulf War. (6)

Although no specific disorder has been identified in GWI veterans, and the etiologic basis and clinical significance of their symptoms remain unclear(6), recent GWI research studies have elucidated the mechanisms of GWI. Our primary objective was to characterize self-reported survey findings among GWI veterans who met our case definition and participated in this study to add additional information to

assumption that omissions happened completely at random. All statistical analyses were performed with SAS software (SAS Institute Inc, 2010), version 9.4 (SAS Institute, Cary, NC).

Results:

Of the 192 participants assessed for eligibility, 104 subjects underwent randomization. Fifty-two were randomized to biweekly treatment and 52 were randomized to waitlist to weekly treatment. Mean age was 48 years in both groups, 13% were female in both groups, and 83% self-reported as white in the biweekly treatment group and 79% self-described as white in the waitlist to weekly treatment group. Baseline characteristics are summarized in Table 1.

[Please insert Table 1 here]

Participants were asked about their concurrent secondary diagnoses. Over 1/3 of participants indicated that they were currently diagnosed with anxiety (36% of the sample), depression (35%) and Post Traumatic Stress Disorder (33%). Other concurrent diagnoses included sleep apnea (28%), Chronic Fatigue Syndrome

consciousness, catastrophizing, depression and anxiety are noted in Table 4.

[Please insert Table 4 here]

Discussion:

Participants in this study were predominately male, white, not Hispanic and the average age was 48 years. Over a third of this sample reported current diagnoses of anxiety, depression, PTSD. Over a quarter of the sample reported concurrent diagnoses of sleep apnea and Chronic Fatigue Syndrome. Diseases of the musculoskeletal system and connective tissue were reported by 75% of the sample, symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified were experienced by 51% of the sample, and Mental, Behavioral and Neurodevelopmental disorders were reported by 31% of the sample. Ratings on standardized instruments were higher than population norms for the SF-36 scale, where 2005-2006 population mean= 50.68 (SD = 14.48) and our sample mean = 67.73 (SD = 23.48) (20). The present study participants also reported differently than

Illness. It is plausible that our sample was 'sicker' than the general Gul War veteran population. Last, we sampled people with GWI who were interested in participating in a trial of acupuncture for GWI. It is plausible we have a skewed sample, as well.

It is clear that veterans of the Gulf War are more likely to experience mental illness such as depression, anxiety and PTSD, as well as physical illnesses and symptoms, as compared to veterans of the same era who did not serve in the Gulf War. Further research is needed to identify safe and effective interventions for veterans of the Gulf War.

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Table 2. Frequency of secondary diagnosis

Secondary Diagnosis	Total N	Yes – I have it currently N (%)	Yes – I had it in the past N (%)	No – I have never had it N (%)	I'm not sure N (%)	Missing N (%)
Fibromyalgia	103	9 (8.74%)	2 (1.94%)	63 (61.17%)	13 (12.62%)	16 (15.53%)
Interstitial Cystitis	103	1 (0.97%)	0 (0%)	73 (70.87%)	10 (9.71%)	19 (18.45%)
Chronic Fatigue	103	28 (27.18%)	8 (7.77%)	40 (38.83%)	14 (13.59%)	13 (12.62%)
Gastroesophageal reflux	103	20 (19.42%)	13 (12.62%)	43 (41.75%)	9 (8.74%)	18 (17.48%)
Sleep apnea	103	29 (28.16%)	3 (2.91%)	44 (42.72%)	10 (9.71%)	17 (16.50%)
Depression	103	36 (34.95%)	18 (17.48%)	36 (34.95%)	6 (5.83%)	7 (6.80%)
Anxiety	103	37 (35.92%)	15 (14.56%)	33 (32.04%)	10 (9.71%)	8 (7.77%)
IBS	103	13 (12.62%)	5 (4.85%)	49 (47.57%)	14 (13.59%)	22 (21.36%)

Table 3. Major Symptoms

2016 ICD-10-CM Codes	Sx 1 Percent (%)	Sx 2 Percent (%)	Total Percent (%)
Mental, Behavioral and Neurodevelopmental disorders	16.5	14.56	31.06
Diseases of the nervous system	2.91	4.85	7.76
Diseases of the eye and adnexa	0.97	0	0.97
Diseases of the ear and mastoid process	0.97	0	0.97
Diseases of the digestive system	1.94	8.74	10.68
Diseases of the musculoskeletal system and connective tissue	43.69	32.04	75.73
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	26.21	25.24	51.45
Injury, poisoning and certain other consequences of external causes	0.97	0	0.97
Unknown	5.83	14.56	20.39
Total	100	100	200

Effectiveness in Clinical Trial Recruitment: An Analysis of Methods used in a Novel Randomized Clinical Trial for the Treatment of Gulf War Illness

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Introduction:

Available data indicates that following the first Persian Gulf War (Operating Desert Shield/Storm, occurring 1990-1991) a quarter of veterans returning were found to experience a symptom cluster, that later became known as Gulf War Illness [1,2]. There are an estimated 100,000 veterans that have GWI. This symptom cluster included chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome and digestive complaints. Additionally, these veterans have reported mood-related psychiatric disorders including depression, anxiety, and post-traumatic stress disorder (PTSD) [3]. GWI illness has been poorly understood with no clearly delineated pathophysiologic process since it was first defined by the Centers for Disease Control (CDC) in 1995. Due to the nature of the disease, the presentation from one patient to the next is often highly variable making successful treatment with conventional pharmacologic strategies.

Methods:

A Department of Defense (DoD) funded randomized-controlled clinical trial was conducted evaluating the effectiveness of acupuncture in the treatment of Gulf War Illness. This was carried out through the utilization of private practice acupuncture offices dispersed throughout

Braintree Ma. The advertisement ran from 12/21/2010-01/14/2011. The dates were chosen in a fashion that would allow veterans who may have been traveling during the holidays to still be exposed to the advertisement and inquire about the study.

Internet – Brief, text only, advertisements were listed on several websites that were strategically chosen for a high likelihood of exposure to Gulf War veterans. These web pages included clinicaltrials.gov, craigslist, clinicalconnections.com, and gulfweb.org. The study was also promoted on the New England School of Acupuncture website.

Veteran specific websites including 91outcomes.com and veteransnewsnow.com also featured study related information on their web pages.

Events:
The study team attended a total of five Yellow Ribbon Post Mobilization seminars to reach veterans returning from deployments. The goal was to reach out to veterans that may have served in the first Gulf War and to spread awareness about the trial. Flyers were also handed out for display in National Guard barracks and the offices of several veteran centered agencies attending these events.

A Disabled American Veterans (DAV) conference was attended in 2011 to increase study awareness in the disabled veteran population.

Flyers, Letters and Post Cards:
The study team utilized 8.5 x 11' flyers printed in color for distribution. These posters featured pertinent information for potential study participants including symptoms of Gulf War Illness, requirements pertaining to deployments (dates, times, locations), and contacts for inquiring about more study information. The flyers were printed in two different formats. The first format was in the form of a letter containing all information for the study. The second format was printed with information and pull-away tabs at the bottom of the flyer that a person could take with them. These tabs had contact information for the study.

Multiple methods were employed to disseminate the flyers. A mass mailing sent to personnel at employment and career centers throughout the Commonwealth (Department of Employment and Training, Commonwealth of Massachusetts.) These centers were chosen to hopefully get information to veterans that may have been seeking employment. It is unknown if these flyers were posted or distributed in any way.

Flyers were also posted in areas felt by the study team to have a high likelihood of being seen by a veteran or the family member/friend of a veteran. These sites included Massachusetts Bay Transportation Authority (MBTA) stations, Veterans of Foreign Wars (VFW) posts, veteran centered agencies and private businesses. These flyers were placed by paid work study students. Flyers were also placed in the registration bags that were distributed at a Healthy Living Expo occuring in the greater Boston area.

Flyers were distributed to all acupuncturists affiliated with the study to display in their offices and to distribute to any individuals who inquired. As the study progressed, flyers were also given to study participants to distribute to their acquaintances and to post in their local communities.

Letters were sent to pain management clinics in the greater Boston area but no responses were ever received.

The recruitment effort with the highest yield for generating study participants was recruitment aimed directly at veterans from the Manpower Data Center information. We were privileged to contact information for 3,390 veterans residing in the Commonwealth of Massachusetts. Of that number, it determined that 3,244 veterans would fit our criteria in terms of location and being able to participate in the study. These 3,244 veterans received postcards and flyers. The second highest source of study participants came from flyers/posters distributed at veteran's associations and agencies.

One study tactic was attempting to have study participants that were having a positive experience with the study recruit other veterans. A total of six study participants were recruited from word of mouth referrals.

Through all recruitment efforts that were employed we generated sixty e-mail inquiries about the study and 225 phone calls. 163 of these inquires made it through to an initial study screening interview (the others were deemed to be disqualified upon initial contact with the study team.) The initial screening process was conducted over the phone and consisted of a checklist that was based on the original GWI diagnostic criteria set forth by the Centers for Disease Control. These criteria included: 1. Deployed to the "Gulf Theater of Operations" as defined by 38 CFR 3.317 which included Iraq, Kuwait, Saudi Arabia, Oman, the Persian Gulf, the Arabian Sea and the Red Sea as well as the airspace above these regions in the years 1990-1992. 2. They have at least two of the symptoms from the three CDC clusters of symptoms. 3) These symptoms have lasted longer than six months in duration. Each symptom cluster must be characterized as "mild-moderate" or "severe" with a least one symptom in each cluster having the designation as "severe."

Information from the phone screening was logged into the Filemaker Database. This database automatically determined the eligibility of each veteran to enter the study based on the inputted information. Eligible veterans were asked if they would like to participate in the study and those who wished to move forward were scheduled for an in person medical screening performed by the study physician. Medical screenings were conducted on one to two Saturdays each month depending on the physician's availability.

Of the 225 initial calls that were placed, 29 veterans declined to participate in the study. The reasons for these included being too far from the study location, lack of reimbursement for travel, and inability to commit from a time standpoint. Of the 163 veterans who went through the pre-screening, 22 were found to be ineligible for the study. Five eligible veterans chose not to move forward with the medical screening. The remaining 136 participants moved on to the medical screening stage of the recruitment process.

15 medical screening appointments that were arranged resulted in a "no-show" by the potential study participant. In these cases, the veteran was contacted by the study team but contact could not be established. 49 veterans went through the study to some degree but were lost throughout the study and could not be reached. The results of the study were a successfully recruited population of 104 subjects (90 men and 14 women.) The average study participant age was 48 years old. 17 people removed consent from the study either for personal reasons.

Discussion:

The most successful recruitment strategy for the study was directed posters/flyers/phone calls for the population we were attempting to recruit. These efforts proved to be far more effective than blindly advertising in public areas. It is also likely (although difficult to quantify) that the

Other Veteran Agency	12
Radio (WRKO)	3
Springfield Republican	5
Stand Down Event	1
Stars & Stripes	1
Unknown	3

a. General Inquiries

Source of Inquiry	**No. of Responses**
Boston Herald	4
Boston Metro	1
Friend	6
Internet Search	1
Manpower Database	65
MBTA	2
NESA	1
Other Veteran Agency	12
Radio (WRKO)	3
Springfield Republican	4
Stand Down Event	1
Stars & Stripes	1
Unknown	3

b. Sources by Study Subject

Title: Matrix analysis of traditional Chinese medicine differential diagnoses in Gulf War Illness

Running title: Chinese Medicine differential diagnoses in GWI

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Target Journal: JACM or PLoS One http://journals.plos.org/plosone/s/journal-information#loc-scope

Introduction

According to clinical and registry programs, 25% of the 700,000 veterans of the first Gulf War are affected by multiple symptoms and co-morbid medical diagnoses that include: chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, post-traumatic stress disorder (PTSD), and other anxiety disorders (Conboy, St John, & Schnyer, 2012). Some first Gulf War veterans have been diagnosed with Gulf War Illness (GWI). GWI presents with difficult symptom presentations, often with complex co-morbid symptoms that consist of fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain. These symptoms are grouped into three symptom clusters by the Centers for Disease Control and Prevention (CDC): fatigability (fatigue 24 hours or more after exertion), mood and cognition (feeling depressed, irritable, anxious, difficulty in concentrating, problems getting to sleep), and musculoskeletal (joint or muscle pain). Overall, veterans diagnosed with GWI are stable (not improving) at 5- and 10-year follow ups (Steele, Sastre, Gerkovich, & Cook, 2012) and no standard of care presently exists (Conboy et al., 2016). There is a need for innovative and effective care of GWI.

Acupuncture has been shown to be effective in treating some of the symptoms of GWI as evident in published research studies, specifically for pain, anxiety, depression, and musculoskeletal disorders. The parent study to this project demonstrated clinically and statistically significant improvement in SF-36 physical and McGill Pain Index scores in the group receiving acupuncture twice per week for six months (Conboy et al., 2016). Preliminary studies indicate that acupuncture may be effective in the management of other complex diseases which share a similar cluster of symptoms (Conboy et al., 2016). The complex diagnostic and treatment process of Chinese medicine (CM), which is tailored to each individual's clinical presentation, can provide an effective framework for evaluating and addressing the complex symptoms presented in GWI. Currently, there are no theoretical CM frameworks for GWI. Our

Frequency counts were tabulated for each differential diagnosis. Matrix analysis was then applied to categorize differential diagnoses according to categorizations of excess, deficient and channel disorders for baseline and 6-months visits. This allowed the detection patterns of consistency and patterns of change in CM differential diagnoses over time.

Matrix Analysis

Matrix analysis is a qualitative data analysis that involves "the crossing of two or more main dimensions . . . to see how they interact" (Miles & Huberman, 1994). Here, we have two dimensions: individual differential diagnoses and categories of excess, deficiency and/or channel diagnoses. These categories are not mutually exclusive and commonly co-occur in clinical practice. Matrices can be descriptive (depicting conditions), outcome-oriented (depicting results or consequences), or process-oriented (depicting dynamics of change) (Averill, 2002). Here we develop both descriptive and process-oriented matrices.

Two coders (LTS and JC) were trained and all participants' data were coded first according to each type of differential diagnosis, and then according to excess, deficiency and/or channel concerns. Cohen's kappa was calculated and the two coders compared any differences, and discussed their interpretations of the coding scheme until agreement was obtained.

Results

The study participants were not statistically significantly different from one another with respect to the following demographics: the mean age was 48.2 in both groups, 13% were

differential diagnosis component at baseline are displayed below in Table 1. Next, each diagnosis was organized according to CM theory into one of three categories. These categories are general or overarching theoretical constructs, and encompass deficiency (e.g., insufficient Qi), excess (e.g., overabundance of Qi, which then may become stagnant) and pathology of the acupuncture meridians and channels (e.g., Qi is stagnant in a channel, causing local pain). The categories and the associated differential diagnoses are:

Deficiency (of Qi, Blood, Yin or Yang)

Excess (of Damp, Heat, Cold, Phlegm, Fire, Yang rising, Wind, Qi stagnation or Blood stagnation)

Channel pathology (Qi & Blood stagnation in the channels, Deficiency of Blood, Yin in the channels, 8 Extraordinary Meridians or Damp Bi Syndrome).

We further analyzed the top 10 of 17 categories with Matrix Analysis, according to frequency. The 10 highest frequency categories are: Qi deficiency, Blood deficiency, Yin deficiency, Qi stagnation, Dampness, Heat, Yang rising, Phlegm, Qi & Blood stagnation and Damp Bi syndrome, as noted in Table 1.

Table 1 Differential diagnosis categories at baseline

Category	N=
DEFICIENCY	
Qi	67
Blood	24
Yin	33
Yang	7
EXCESS	
Damp	25

Excess + Deficiency + Channel	24
Deficiency + Channel OR excess + channel (NOT excess and deficiency)	16
No data available	2

One category of differential diagnosis

Twelve individuals had a single category of differential diagnosis. Of the participants with a single category of diagnosis, Qi deficiency was the most common (6 instances), followed by Yin deficiency, Yang rising, Damp Bi syndrome (2 instances), Yang deficiency, Blood deficiency, Qi stagnation and channel Qi stagnation (1 instance each).

Two categories of differential diagnoses

Forty participants' differential diagnoses were a mixture of excess and deficiency. The most frequent differential diagnosis combination was Qi deficiency & Qi stagnation (n=12 cases), followed by Qi & Yin deficiency with Qi stagnation (n=3 cases), Qi deficiency with Damp & Heat (2 cases), Qi deficiency with Qi stagnation, Damp & Heat (2 cases), Blood deficiency with Qi stagnation, Damp & Heat (2 cases), Qi & Blood deficiency with Qi stagnation, Damp & Heat (2 cases), Qi, Blood & Yin deficiency with Qi stagnation & Yang rising (2 cases). Fourteen (14) other combinations were each seen in one case only. One case could not be summarized in the table (see note).

Three categories of differential diagnoses

Twenty-four participants presented with differential diagnoses at baseline categorized by co-occurring excess and deficiency and channel pathology. These participants were the most complex in their differential diagnoses, with multiple co-occurring categories of disharmony. Please refer to Table 3.

Qi, Yin xu; Qi stag, Damp, Heat; **Qi/Blood channel**	1
Qi, Yin xu; Qi stag, Heat, Yang rising; **Damp Bi**	1
Qi, Blood xu; Qi stagnation, Damp, Heat; **Qi/Blood channel**	1
3 deficiency, 1 excess, 2 channel (1 case)	
Qi, Blood, Yin xu; Damp; **Qi/Blood channel, Bi syndrome**	1
3 deficiency, 3 excess, 1 channel (1 case)	
Qi, Blood, Yin xu; Heat, Yang Rising, Phlegm; **Qi/Blood channel**	1
NOTE: n=4 cases' data not summarized in Table 6 as one of their differential diagnosis categories was not a top 10 in frequency.	

Matrix analysis at 6-months

Differential diagnoses were evaluated at 6-months and classified according to excess, deficiency and channel problems. Please refer to Table 4. A within-person analysis was also performed and 25 participants' diagnoses changed from one category to another (e.g., co-occurring excess and deficiency at baseline and one category only at 6-months), 51 participants' category was the same at baseline and at 6-months, and 18 participants could not be categorized due to missing data.

Table 4. Baseline and 6-month differential diagnoses

Category	Baseline Frequency N (%)	6-month Frequency N (%)	Descriptive changes
Only 1 category (e.g., excess or deficiency or channel)	12 (13%)	15 (16%)	Slight increase
Excess + Deficiency	40 (43%)	36 (38%)	Slight decrease

stagnation in the channels and Damp Bi syndrome. Here, participants exhibited a superficial excess condition, with underlying deficient symptoms, which leads to symptoms within the channels of pain (qi & blood stagnation and damp-bi syndrome).

It is interesting to note that participants in the bi-weekly acupuncture treatment group demonstrated clinically and statistically significant improvement in SF-36 Physical and McGill Pain scores (Conboy et al., 2016) and yet the overall trend is stability in differential diagnoses. In CM theory, a concept about root and branch presentations may help explain why symptoms improved, but differential diagnoses did not always change. It is plausible that the differential diagnoses were reflecting root presentations, meaning very stable individual constitutions. This root is compared to branch diagnoses, which are reflective of emergent or acute conditions. For this reason, it is plausible that while participants' symptoms improved, their differential diagnoses were rather stable, perhaps reflective of root constitutions and not branch symptoms.

Limitations of this study include an increase in missing data at 6-months. This made it impossible to categorize 18 participants' differential diagnoses. Additionally, while the parent study was adequately powered to detect clinically and statistically significant change in primary and secondary outcomes, the analyses here demonstrated very small N in several classification categories. Due to the small sample size, the matrix analyses needs to be replicated with a larger GWI acupuncture study in order to further validate the differential diagnosis co-occurrences for GWI.

Since GWI is a unique and novel disease state and treatment with acupuncture has only initially been evaluated (our parent study), the development of a CM treatment manual is warranted. The treatment manual could utilize the differential diagnose framework developed here (deficiency, excess, channel concerns) as a basis for symptom presentations that are most likely to occur in patients with GWI. A theoretical CM pathological foundation could also be explored as to the causes of qi, yin, yang, and blood deficiency or excess in GWI patients.

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The Importance and Determination of Dosage in Acupuncture Treatment

Introduction:

Gulf War Illness (GWI), or chronic multisymptom illness (CMI), is a complex illness characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain, which are unexplained by physical and laboratory examinations. There is no standard of care treatment for this syndrome at this time. First defined by the Centers for Disease Control and Prevention (CDC) after the first Gulf War[i], it is commonly seen with a highly individualistic presentation, associated with clusters of symptoms and co-morbid medical diagnoses, including chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, posttraumatic stress disorder (PTSD), and other anxiety disorders[ii,iii]. It has been shown to be remarkably stable at 5- and 10- year follow-ups[iv,v]. Of the 700,000 service personnel deployed to the Persian Gulf, at least one-fourth of the veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990-1991) are affected by GWI[vi,vii]. GWI is twice as prevalent in deployed veterans, and seen in 15% of non-deployed veterans[viii]. CMI symptoms have been studied in cohorts of veterans in the United Kingdom, Canada, and Australia. The etiology of CMI is still unknown, and hypotheses involving exposures to vaccines, medications, pesticides, chemical munitions, and inhalation of depleted uranium dust and smoke from burning oil fields, have all been investigated.

In 2016, our study team published results of The Effectiveness of Acupuncture in the Treatment of Gulf War Illness. This pragmatic Randomized Clinical Trial tested the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community; practitioners had at least 5 years of experience plus additional training provided by the study. Veterans with diagnosed symptoms of Gulf War Illness were randomized to either six months of biweekly acupuncture treatments (group 1, n = 52) or 2 months of waitlist followed by weekly acupuncture treatments (group 2, n = 52). Measurements were taken at baseline, 2, 4 and 6 months. The primary outcome is the SF-36 physical component scale score (SF-36P) and the secondary outcome is the McGill Pain scale. Of the 104 subjects who underwent randomization, 85 completed the protocol (82%). A clinically and statistically significant average improvement of 9.4 points ($p = 0.03$) in the SF-36P was observed for group 1 at month 6 compared to group 2, adjusting for baseline pain. The secondary outcome of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points ($p = 0.04$) compared to group 2. (Conboy, 2016).

This parent trial used individualized acupuncture protocols. To better understand effective dosage of acupuncture for GWI symptoms, this paper considers the relationship between actual dose and symptom relief, as well as other factors related to actual dose which may have clinical importance.

Background:

Acupuncture is a widely used and increasingly studied treatment modality, yet substantial disagreement remains about its effectiveness and how it should best be utilized. Great progress has been made in elucidating the physiological effects of Acupuncture treatment, but there is still much that is only partially or poorly understood. (Zhuang, Xing, Li, Zeng, & Liang, 2013) There

Acupuncture as it is actually practiced clinically is an individualized treatment process, with neurophysiological dosage determined by the patient's condition on the day of treatment. (O'Connor & Bensky, 1981)(Maciocia, 2008)(Langevin & Schnyer, 2017)
Since the neurophysiological dose will vary between patients and from treatment to treatment, even patients who have been treated for the same condition using the same cumulative dosage of treatments, are unlikely to have received the same neurophysiological dosage of Acupuncture. This situation can lead to confusion as to how total Acupuncture dosage over time should be compared across patients.

However, if a consistent diagnostic and treatment process is followed in determining optimal neurophysiological dosages for each patient, meaningful comparisons about cumulative dosage and treatment effectiveness can be made. This approach has been followed in a number of recent studies. (Schnyer, Iuliano, Kay, Shields, & Wayne, 2008) (Conboy, St John, & Schnyer, 2012) (Conboy et al., 2016) In all of these studies the participating Acupuncture practitioners were trained and experienced in using the same treatment protocols, and they agreed to use the same specifically outlined diagnostic procedures in deciding how to treat their patients. Within this constraint they were free to choose whatever specific neurophysiological dose that they felt was appropriate for a patient on a given day. This process reflects the way that Acupuncture is actually practiced in most clinics. Studies designed in this way are both naturalistic and rigorous, conforming to the STRICTA recommendations for Acupuncture controlled trials. (MacPherson et al., 2002)

Once the issue of neurophysiological dosage is addressed, it becomes meaningful to compare cumulative dosages. Currently there is surprisingly little data about the importance of cumulative dosage in determining Acupuncture treatment effectiveness. A literature review was done by the author (BAS) in September 2017 searching Medline, Google scholar, and Cochrane databases using the keywords 'Acupuncture therapy' (MeSH term) AND 'frequency,' 'dosage' and 'schedule'. No comprehensive studies or reviews of the significance of cumulative dosage across all types of Acupuncture treatment were found.

However, there are numerous studies containing some cumulative dosage data, and some of these studies include data comparing the effectiveness of different cumulative dosages for specific conditions. A 2005 study investigating Acupuncture treatment for fibromyalgia indicated that cumulative dosage was more important than correct Acupuncture point needle placement in determining treatment success, and that 3 treatments weekly provided greater pain relief than 1 treatment weekly. (Harris et al., 2005) A large study investigating Acupuncture treatment of chronic pain indicated that both number of needles used, and total cumulative dosage were significant; with more needles and more treatments associated with greater pain relief. (MacPherson et al., 2013) Indeed our 2016 study investigating the treatment of Gulf War Illness with Acupuncture found that treatment twice weekly was more effective than treatment once weekly. (Conboy et al., 2016)

Armor and Smith did a literature review to specifically investigate the relationship between Acupuncture treatment dosages and pain relief outcomes in treating dysmenorrhea. Summarizing the results of 11 trials, they concluded that treatment timing, needle stimulation, and number of needles all appeared to be significant factors in influencing pain reduction. Treatment started

of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points ($p=0.04$) compared to group 2.[x]

This current analysis considers the relationship between actual dose, as measured by practitioner treatment records, and our main outcomes using Pearson correlation. Secondly, considering dose in a manner similar to adherence, we consider if there is a relationship between actual dose and other physical and psychosocial health markers at baseline, as well as quality of relationship with practitioner.

When considering which variables to consider in the prediction of adherence, we first thought of confidence, and/or satisfaction with treatment. But in this sample there is very little variation in these variables and all subjects reported high confidence and satisfaction. Thus instead we considered the baseline variables of health behaviors (smoking, & drinking behavior), anxiety (Beck), depression (Whitely), social support (ISEL), mood (POMS) and reported social networks. We also considered the patients' viewpoint on the quality of the patient-practitioner relationship at the first measurement timepoint of 2 months; we did not measure relationship at baseline because the patient-practitioner relationship had not started yet.

We considered the relationships between actual dose and the variables above with descriptive statistics (mean, variance) and simple Pearson correlations for continuous variables and Spearman correlation for categorical variables.

Results:

Actual dose/number of treatments across the whole sample ranged from a minimum of one treatment to a maximum of 49, with a mean of 23.99 (SD 12.98). As expected the treatment arm assignment is related to actual dose ($r=0.73$, $p<0.001$).

Figure 1:Frequency distribution of actual dose by treatment group assignment.

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Figure 2: Relationship of Actual Dose and pain resolution by 6 months. Change scores above zero indicate worsening reported pain; scores below the line indicate improvement in pain.

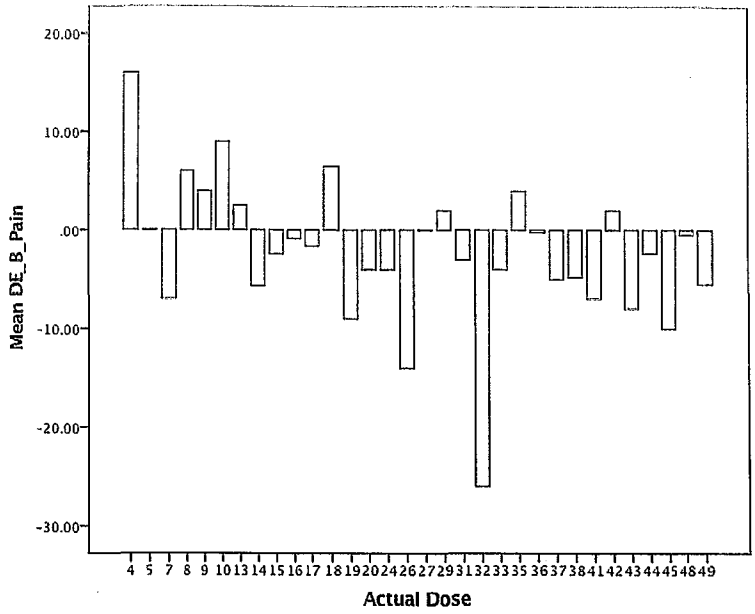
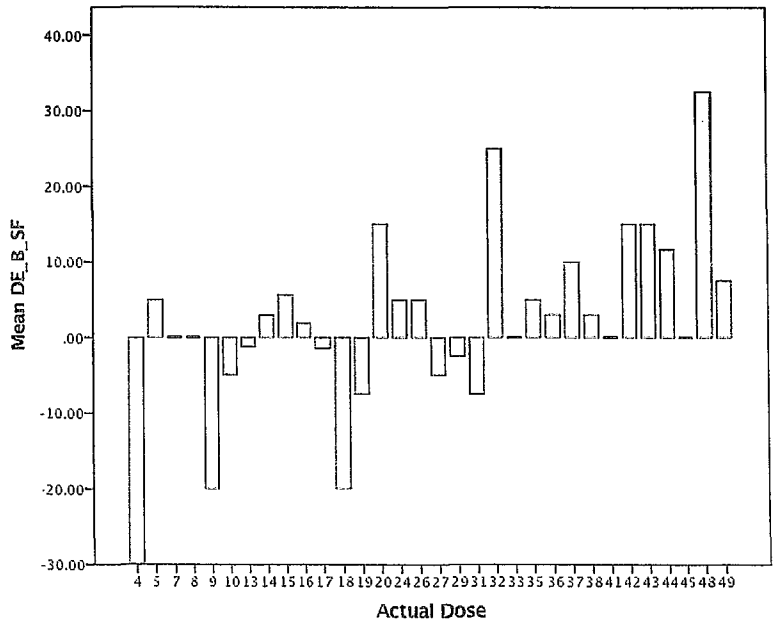


Figure 3: Relationship of Actual Dose and improvements in physical function by 6 months. Change scores below zero indicate worsening reported function; scores above the line indicate improvement in function.



We also considered the relationship of Actual Dose and pain resolution by 6 months. (Figure 2). It appears that in this sample symptom improvement most clearly begins at 18 treatments. In consideration of the relationship of Actual Dose and physical function by 6 months. (Figure 3), it appears that in this sample symptom improvement most likely occurs after 33 treatments.

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Introduction:

The Therapeutic Alliance (TA) is the relationship created between patient and practitioner, which is also recognized as therapeutic relationship, therapeutic bond, treatment alliance, helping alliance, or working alliance. It is considered vital to treatment effectiveness in general psychotherapy (1-3) and is one of the most often studied subjects within concurrent clinical psychology (4-6). TA is rooted in brain coupling of patient and therapist (1) and purported to create the necessary climate and conditions in which other intervention contents can be successfully delivered by the therapist and absorbed by the patient (3,7-10). However, traditional TA is abraded by rapid pace of medical advances in addition to specialization and super-specialization (11)

TA encompasses three major elements: (i) tasks, the collaborative endorsement of the intra therapy activities (includes an understanding of what is required of each of the parties in the performance of these tasks, and an appreciation of the relevancy of the tasks to the therapy process); (ii) goals, the mutual agreement and valuing of the outcomes of the therapy; and (iii) bonds, that encompass the complex elements of attachment between the patient and clinician such as trust, empathy, personal liking and valuing (12).

Clinical studies have been reported by a meta- analysis that, the practitioner patient relationship has a significant effect on multiple healthcare outcomes including quality of life, psychological problems (e.g. anxiety, depression), re-consultation rate, smoking quit rate, pain relief, blood pressure, weight loss, etc. (13-26). TA consistently has shown to predict outcomes in psychotherapy (27-31) but studied to a lesser extent in other health fields such as Traditional Chinese Medicine (TCM) (32). TCM is a form of Eastern Medicine that is over 2000 years old. TCM practitioners utilize various techniques like acupuncture, tui na, nutrition, moxabustion, and tai qi to treat the mind and body of patients. Acupuncture being the most commonly used in America. In TCM, TA is a form of artistry, called the "Penetrating Divine Illumination" and is "the refinement of the physician's art." It is a transformative healing state between the Qi of physician and patient, which resonates at a spiritual level. Empathetic dialogue and assessment create the Penetrating Divine Illumination, which becomes part of the treatment or intervention. (33) Acupuncture, similar to other forms of health care areas, occurs in a specific setting that can affect clinical outcomes, the patient-acupuncturist relationship is one variable of this setting (34). Studies conducted in this area indicated TA as a predictive factor for therapeutic outcomes. Beneficial outcomes as a result of relationships between practitioners and clients in the field of

6 month period, N=36 for ISEL_Sum, N=37 for CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum. For treatment group 2, where treatment was delayed by 2 months and then continued weekly for the remaining 4 months, N=32 for ISEL_Sum, CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum.

Statistical Analysis

Average WAI scores were calculated for each participant and practitioner and results for each factor were graphed by patient-practitioner dyad over time. We next calculated change in concordance for each dyad from baseline to 6 month endpoint. Linear regression models are used to measure the influence of degree of change in concordance on the change in outcomes of pain and physical function.

To assess whether a more congruent patient and practitioner relationship was indicative of superior treatment outcomes, Working Alliance Inventory-Short Revised (WAI-SR) difference scores between patients and practitioners were utilized and compared to clinical outcomes for ISEL_Sum, CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum. WAI-SR difference scores were reviewed for similarity of agreement in tasks, goal and the formation of an affective bond during treatment.

The correlation coefficients that were statistically significant ($p<0.05$) included:

Treatment group 2: CDEP_Psychological_Anxiety_1Sum Δ with PP_ Δ_Bond ($p=0.009$). Correlation coefficient analysis for ISEL_Sum and CDEP_Psychological_Anxiety_Sum varied, however, no stastistical significance was detected (Table 1).

Table 1. Pearson correlation coefficient per treatment group

Treatment Group 1						
Clinical Outcome	PP_Δ_Task	Sig. (1-tailed)	PP_Δ_Bond	Sig. (1-tailed)	PP_Δ_Goal	Sig. (1-tailed)
ISEL_1Sum (N=36)	-0.099	0.282	0.105	0.272	0.06	0.364
ΔISEL_Sum (N=36)	-0.051	0.385	-0.012	0.472	0.149	0.193
CDEP_Depression_1Sum (N=37)	-0.16	0.173	-0.21	0.106	-0.071	0.338
ΔCDEP_Depression_Sum (N=37)	0.029	0.432	0.035	0.418	0.085	0.309
CDEP_Psychological_Anxiety_1Sum (N=37)	-0.08	0.318	-0.205	0.112	-0.107	0.265
ΔCDEP_Psychological_Anxiety_Sum (N=37)	0.147	0.193	-0.02	0.453	0.117	0.246

Treatment Group 2						
Clinical Outcome	PP_Δ_Task	Sig. (1-tailed)	PP_Δ_Bond	Sig. (1-tailed)	PP_Δ_Goal	Sig. (1-tailed)
ISEL_1Sum (N=32)	-0.21	0.125	-0.081	0.33	-0.196	0.141
ΔISEL_Sum (N=32)	0.092	0.308	0.114	0.267	-0.037	0.421
CDEP_Depression_1Sum (N=32)	-0.044	0.406	-0.062	0.367	0.107	0.281
ΔCDEP_Depression_Sum (N=32)	0.114	0.267	-0.142	0.22	0.009	0.481
CDEP_Psychological_Anxiety_1Sum (N=32)	-0.214	0.12	-0.418	0.009	-0.103	0.287
ΔCDEP_Psychological_Anxiety_Sum (N=32)	0.117	0.262	-0.042	0.41	0.128	0.243

The correlation coefficients that were statistically significant ($p<0.05$) where:

Treatment group 1: Δ SF-36 Goal ($p=0.046$)

Treatment group 2: Δ SF-6 Task ($p=0.042$) and Δ SF-36 Goal ($p=0.027$) (Table 2).

Table 2. Pearson correlation coefficient per treatment group

Treatment Group 1						
Clinical Outcome	Task	Sig. (1-tailed)	Bond	Sig. (1-tailed)	Goal	Sig. (1-tailed)
Δ SF-36 (N=37)	-0.166	0.162	-0.269	0.054	-0.281	0.046
Δ McGill (N=37)	0.000	0.499	-0.221	0.095	0.044	0.399

Treatment Group 2						
Clinical Outcome	Task	Sig. (1-tailed)	Bond	Sig. (1-tailed)	Goal	Sig. (1-tailed)
Δ SF-36 (N=32)	-0.309	0.042	-0.158	0.195	-0.345	0.027
Δ McGill (N=31)	0.126	0.250	0.144	0.220	0.062	0.371

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PLOS | ONE

RESEARCH ARTICLE

The Effectiveness of Individualized Acupuncture Protocols in the Treatment of Gulf War Illness: A Pragmatic Randomized Clinical Trial

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Abstract

Background

Gulf War Illness is a Complex Medical Illness characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain affecting veterans of the first Gulf War. No standard of care treatment exists.

Methods

This pragmatic Randomized Clinical Trial tested the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community; practitioners had at least 5 years of experience plus additional training provided by the study. Veterans with diagnosed symptoms of Gulf War Illness were randomized to either six months of biweekly acupuncture treatments (group 1, n = 52) or 2 months of waitlist followed by weekly acupuncture treatments (group 2, n = 52). Measurements were taken at baseline, 2, 4 and 6 months. The primary outcome is the SF-36 physical component scale score (SF-36P) and the secondary outcome is the McGill Pain scale.

Results

Of the 104 subjects who underwent randomization, 85 completed the protocol (82%). A clinically and statistically significant average improvement of 9.4 points ($p = 0.03$) in the SF-36P was observed for group 1 at month 6 compared to group 2, adjusting for baseline pain. The secondary outcome of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points ($p = 0.04$) compared to group 2.

offices in communities where veterans work and live. We began recruitment of subjects and practitioners with a catchment area of 30 miles from our research study offices, and widened our catchment area with increasing study duration.

Veterans with Gulf War Illness were randomized to either (1) acupuncture treatment twice per week for 6 months or (2) the wait-list comparison group consisting of usual care from baseline for 2 months, followed by weekly treatments for 4 months. We chose an active control group to maximize internal validity while allowing us to gather preliminary data on minimal effective treatment dose. The two month wait time allowed us to judge if GWI symptom presentations are stable in our sample, as has been shown in other GWI samples [1,5]. Our treatment schedule duration, dose, and specific Chinese Medicine techniques employed are based on the clinical experience of our expert practitioners, and informed by literature review. Details of the protocol and implementation were determined before the trial began via focus groups with senior acupuncture faculty at the New England School of Acupuncture.

The New England Institutional Review Board approved this research protocol on September 4, 2009. All human participants gave written informed consent. All of our study processes were approved and oversight is provided by: 1) The New England Institutional Review Board (http://www.neirb.com/), 2) United States Army Human Research Protection Office (https://mrmc-www.army.mil/rodorphrpo.asp). The study operated as planned between September 2009 and January 2013. Recruitment began immediately, and ran until July 2012. We initiated the clinical trial registration process before recruiting subjects, and registration was completed before we began to analyze the data. We confirm that all ongoing and related trials are registered. Please see S2 File. CONSORT Checklist.

Recruitment. We recruited via local advertisements and direct mailing to veterans of the first Gulf War drawn from the Defense Manpower Data Center (http://www.virec.research.va.gov/Non-VADataSources/DMDC.htm). Because the demographics of GWI veterans are unpublished, we did not know if there was a sufficient population near our study offices from which to draw our sample. Thus we designed the study to include treatment sites within a 100-mile radius of our study offices, and incorporated a mechanism to add treatment sites within that radius in areas where GWI veterans were found clustered. Thirty treatment sites were utilized. This design has the added benefit of allowing veterans to attain treatments near where they live and work, a technique that may have improved adherence.

Eligibility. All subjects met the illness definition of Gulf War Illness as determined by responses on the Gulf War Illness Symptom Checklist [5] and the inclusion/exclusion criteria set forth in the federal definition of Gulf War Illness as used for the Gulf War Registry (Please see Box 1). Subjects needed to pass through two eligibility screenings. First, a research assistant conducted a prescreening by phone, querying potential subjects about their illness and symptom experience. Second our study physician (MG) used the same criteria to complete an in-person medical screening. Our two-stage informed consent process included 1) verbal informed consent requested prior to the initial telephone screening, and 2) written informed consent administered in person, at the start of the screening visit. Please see Fig 1. An unblinded member of the study staff (LC) with no additional patient contact enrolled subjects. Study outcomes data were collected by electronic interface at our outpatient clinic. In fewer than 10% of cases, due to participants' time constraints, participants were allowed to take their surveys home to fill out, and then mail back. During this screening visit, participants chose their future treatment practitioner from a list of practitioners convenient for them.

Group assignment and interventions. Collaborator RD randomly assigned participants to the two study arms using permuted block randomization with variable block sizes and assignments provided in sequentially numbered opaque sealed envelopes. After baseline evaluation and consent, a member of the study staff without involvement in data collection with

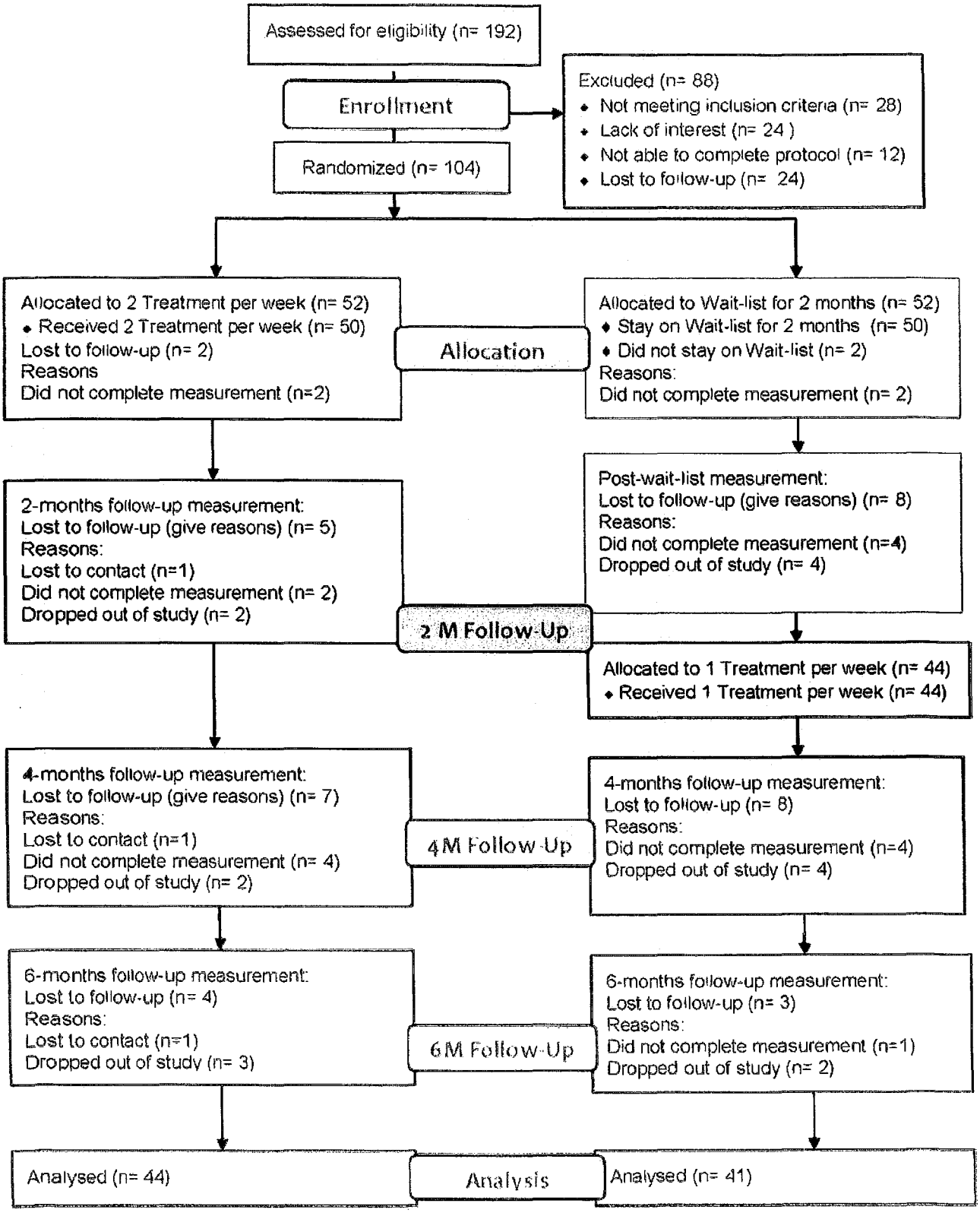

Fig 1. PLOS CONSORT Flow Diagram: Diagram of Screening, Randomization, and Follow-up.

doi:10.1371/journal.pone.0149161.g001

Sample size

Our sample size was calculated to allow detection of clinically meaningful differences between treatment groups. Previous acupuncture research using our main outcome, the SF-36, in pain conditions [27,28] show a consistent standard deviation of 20 points in the SF-36 P for both baseline values and change scores. Sixty individuals per group (total n = 120) would offer us a power of 80% to detect the difference between groups of 7 points. Using Cohen's d estimation of effect size [29] a sample size of 60 would allow us to see a moderate effect. In further support of our main outcome, a 7.8-unit improvement has been estimated clinically relevant for patients with similarly serious conditions [30,31]. We estimated a dropout rate of 10%.

Statistical analyses

In our original proposal to the funder, to protect our main outcome from possibly large attrition, we proposed to initially test mean differences between groups following 2 months of treatment using Student's t-tests at an alpha = 0.05. Using this strategy, we observed a mean reduction in SF-36 for Group 1 of 0.32 versus a reduction 4.53 for Group 2 (p = 0.22).

Of more interest to the study team is what changes might be seen after the clinically informed 6 month treatment window. This 6 month analysis is done by author TG and investigates whether those subjects assigned to biweekly acupuncture experienced differences in the SF-36P score over follow-up compared to those subjects who received weekly acupuncture following a 2-month delay. To assess potential differences, generalized estimating equation (GEE) models were fit in order to account for the correlation induced by repeated measurements on each subject. Under the assumption that baseline McGill pain is prognostic for SF-36P over time, model adjustment was made for baseline pain to increase precision in the estimated parameters.32 Eight subjects did not report this baseline measurement and, under the assumption that missingness was completely at random, these subjects were not included in the analysis set. In summary, we estimated the GEE model

$$SF36 = \beta 0 + \beta 1 Itx + \beta 2 It^2 + \beta 3 It^4 + \beta 4 It^6 + \beta 5 \rho + \beta 6 Itx It^2 + \beta 7 It^4 + \beta 8 Itx It^6$$

where *Itx* denotes an indicator for biweekly acupuncture; It^2,It^4,*and* It^6, are indicators for months 2, 4, and 6; ρ denotes baseline pain; and time was coded categorically to reflect suspected nonlinearities.

As a secondary analysis, the McGill pain score was similarly assessed for differences over time by treatment status. The time trend for the GEE fit was modeled categorically to account for nonlinear trends. The pain model was not adjusted for additional covariates, since no strongly prognostic variables were assumed to have been measured. All GEE models were fit using the software package geepack [33] in R version 3.1.1 under an exchangeable working correlation structure.

Results and Discussion

Study Population

Recruitment began in July of 2010 and the final follow-up visit was completed in January 2013. Please see Table 1 for baseline demographics and Fig 1. for study flow.

Outcomes

Of the 104 subjects who underwent randomization 103 completed at least one measurement timepoint, yielding 99.0% of data for analysis. However, 8 subjects are missing baseline pain data, yielding 95/104 = 91.3% in the analysis set. General Estimating Equations were used to

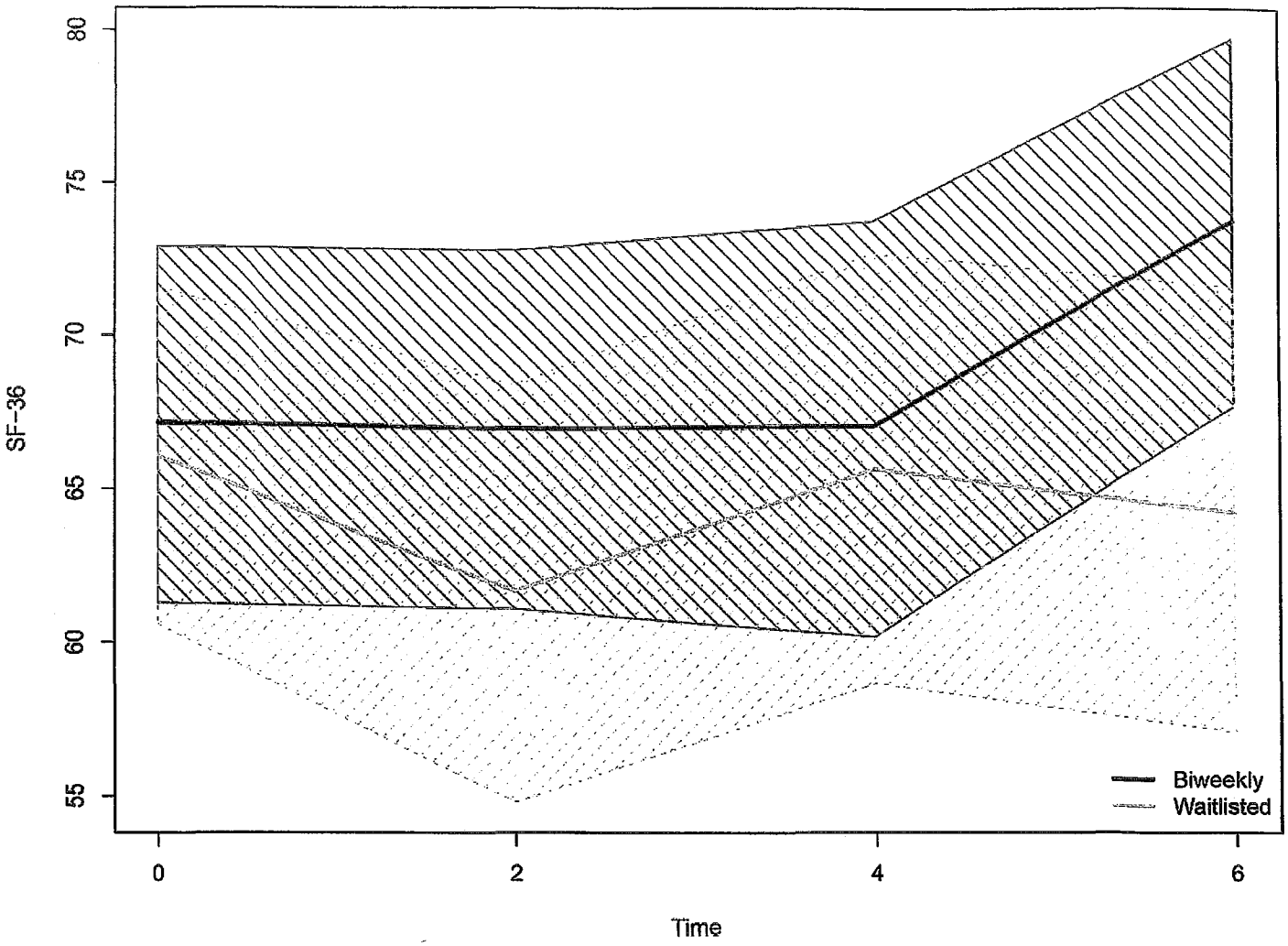

Fig 2. Summary of model-based simulations[xxxiii] of changes in mean SF-36P at 4 measurement timepoints. Scores moving in the positive direction indicate improvement. Scores moving in the positive direction indicate improvement.

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the estimated value and 95% confidence intervals. Tables 3 and 4 offer estimates and accompanying statistical values from the GEE modeling of the two outcomes.

Participants reported high usability of acupuncture with 96% of the veterans (averaged across both groups and over all time points) reporting confidence in recommending acupuncture to a friend or family member (or at least a 3 on a five point scale from "Very Confident" to" Not Confident"), 98% reporting that the acupuncture experience was at least pleasant (or at least a 3 on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant"), 97% reporting that their relationship with their practitioner was pleasant (or at least a 3 on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant") and 96% reported that the acupuncture treatments were logical for them (or at least a 3 on a 6 point scale of "Very Logical" to "Not Logical at All"). The trial had only two adverse events: (1) subject in biweekly treatment group reported pain on needling, (2) subject in weekly treatment group reported suicidal thoughts, which study staff followed up with additional medical oversight.

Table 4. Estimates and accompanying statistical values from the GEE modeling of the outcome McGill Pain scale.

McGill	Estimate	Std Err	95% CI	Wald X2	p-value
Intercept	29.65	1.28	(27.15, 32.15)	539.82	<0.001
Biweekly	0.02	1.73	(-3.38, 3.42)	0.00	0.99
Month 2	1.82	1.06	(-0.26, 3.89)	2.93	0.09
Month 4	-0.31	1.41	(-3.07, 2.45)	0.05	0.82
Month 6	-0.20	1.35	(-2.84, 2.44)	0.02	0.88
Biweekly * Month 2	-2.35	1.29	(-4.88, 0.19)	3.29	0.07
Biweekly * Month 4	-2.56	1.81	(-6.11, 0.99)	2.00	0.16
Biweekly * Month 6	-3.59	1.71	(-6.94, -0.25)	4.43	0.04

doi:10.1371/journal.pone.0149161.t004

Discussion

This study supports the use of individualized acupuncture treatments for the management of GWI symptoms. Our results are in concordance with numerous other studies indicating that acupuncture is a widely available, safe, effective, and cost-effective option for the treatment of other diseases and syndromes with similar presentations to GWI [35] with high usability in veteran populations [36]. Given this research, it is likely that acupuncture treatment may be an effective, safe, low-cost treatment option for our returning military as well as civilian populations impacted by chronic multi-symptom illness and its co-morbidities.

The mechanisms of acupuncture in the treatment of GWI are unknown, which supports our choice of a low-constraint design. This naturalistic RCT includes individualized protocols, a clinically supported length and dose of treatment, and a wait list control. Data from our wait list arm (Table 2) indicates that symptoms are stable, as has been shown in published 5- and 10- year follow-ups [1,5]. The design aspect of the wait list group eventually receiving weekly acupuncture offers us data to begin to answer questions of minimal dose and satisfies ethical concerns allowing all subjects to receive treatment during the study. A sham acupuncture control arm was not used due to published indications that such sham interventions are effective and thus not appropriate controls; very high quality evidence of this is now available [37,38].

We chose a pragmatic design, and used practitioners in the community, to facilitate adherence and test the use of extant practitioners. Our positive results support the referral of GWI veterans to acupuncture treatments. Our low side effect rate mirrors that of the published literature that acupuncture is safe when provided by professionally trained practitioners [39]. Serious adverse events are extremely rare. In a systematic review of 12 prospective studies scrutinizing over a million treatments, the very low risk of serious adverse event, mostly trauma from needle puncture or infection, was estimated at 0.05 per 10,000 treatments, a risk below that of many common medical treatments [40]. Acupuncture is well tolerated, safe and effective in the management of Gulf War Syndrome. The inclusion of acupuncture in the routine management of this intractable condition is warranted.

Limitations and Future Directions

Our results suggest that 2 months of biweekly acupuncture is not sufficient to affect the outcome of physical function (as measured by the SF-36P), but pain scores (as measured by the McGill Pain Scale) did show group improvement as early as the first follow-up (2 months). These findings underscore the need for more dosage studies to determine the most therapeutic level of treatment for different illness presentations. Currently, we are conducting secondary data analyses exploring the effectiveness of acupuncture treatment on different subtypes of GWI to help treatment providers apply the best protocols for this complex illness. The team is

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Dr Lisa Conboy

Appendix B

Statement of Work

Statement of Work

This project is a program evaluation of the Congressionally Directed Medical Research Program funded project "The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" (W81 XWH). This single-blind randomized controlled clinical trial evaluated the effectiveness of individualized acupuncture treatment on subjects' overall health and disease burden.

Objectives: This current application has two objectives: 1) Gather follow up data from our veteran participants on current symptom levels and use of services to explore the long-term effects of an acupuncture treatment program, 2) Develop suggestions for how an acupuncture program may be implemented using the viewpoints of multiple stakeholders.

Tasks:

1. **Create program evaluation documents (Month 1):** Dr Conboy will finalize the survey instrument and focus group agenda. These materials will be circulated to all other study staff for feedback. Suggestions and edits will be made for submission to the IRB.
2. **Train research assistant, and review goals with consultants (Month 1-2)**: Once funding is achieved the team will have a face-to-face meeting (using Skype for long-distance members) to review program goals. Follow-up group e-mails will solidify our process.
3. **IRB Review (Month 1-3):** The protocol will be submitted to the IRB as soon as funding is approved. This approval should take no more than a month. Review will take place at our contracted IRB, the New England IRB (www.neirb.com). Review by the HRPO will also be submitted and completed.
4. **Program Evaluation (Month 4-29):**

Task 4.1 Program evaluation with study subjects: Collect (Month 4-10)

All of the subjects who participated in the parent trial will be mailed an IRB approved survey which: (1) repeats the survey questions administered in the parent study, (2) asks additional questions about subjects' study experiences, use of health services since the study, and requesting feedback and suggestions for program and treatment improvement. Subjects' participation will be requested up to three times (by email, mail, and phone) and subjects can opt out of participation at any time. The mailing will also ask for the subject's interest in participating in a focus group with multiple stakeholders to help design the best acupuncture protocols and program for veterans.

Task 4.1.2 Enter subject surveys into electronic database format. Data will be double entered with appropriate accuracy checks (see Attachment 8: Data Management).

Task 4.1.3 Tabulate subject surveys for use in program evaluation meetings (below Task 4.2) Subjects' responses to survey questions of symptoms, reported beliefs about acupuncture, study experiences, and use of acupuncture since trial completion will be tabulated.

Task 4.1.4 Analyze responses to subject symptom surveys. Paired Student's t-tests will be used to compare subjects' symptom reports since trial completion to the present day to initially determine the long-term effectiveness of acupuncture. The eventual manuscript for publication will utilize regression equations and consider control variables such as age, time since study completion, and baseline symptom levels.

Task 4.2 Program evaluation with multiple stakeholders (Month 11-22)

Assemble team members. Named members include: (1) Joe Chang Lic Ac, an acupuncturist with experience working in military settings; (2) Marc Goldstein MD, a physician at the VA in Boston MA who was the medical screener for the parent project; (3) Meredith St John Lic

STATEMENT OF
WORK – January 12, 2015
PROPOSED START DATE August 1, 2015

Site 1: New England School of
Acupuncture
150 California Street/Newton, MA
PI: Lisa Conboy

Specific Aim 1(specified in proposal)	Timeline	Site 1
Aim 1 Survey veteran participants for their current symptom levels, and use of acupuncture and other services	Months	
Create program evaluation documents	1	Dr Conboy
Train research assistant, and review goals with consultants	1-2	
IRB Review/ HRPO/ACURO Approval	1-3	
Survey participants of parent trial	4-29	
Aim 2 Conduct effectiveness research/program evaluation of our acupuncture treatments and study design from the viewpoint of multiple stakeholders:		
Survey participants of parent trial as described in Aim 1	4-29	Dr Conboy
Conduct Focus groups	11-29	
Conduct Delphi process	18-29	
The product of Delphi analysis is a document of concrete recommendations for implementing an acupuncture treatment program in both VA and community settings.	29	

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Dr Lisa Conboy

Appendix C

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